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St. Paul, Minnesota 55102

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LC Test Method No.: LC910300  
Name: Determination of  
Peroxyoctanoic and Octanoic Acids  
Supercedes: New  
Effective Date: 10-18-91  
Page 1 of 7  
Written By: L.M.Rue

**TEST METHOD****LC Test Method No. LC910300**

**Purpose:** To determine the amount of Peroxyoctanoic acid and Octanoic acid in KX6049 liquid sanitizer product (178.1010) by HPLC.

**Scope:** Applicable to KX6049 liquid sanitizer product.

**Principle:** Reversed phase liquid chromatography is used to determine the amount of peroxyoctanoic acid and octanoic acid in the KX6049 product by Diode Array UV detection and comparison of peaks using an external standard.

**Instrument:** Any equivalent instrument modules may be substituted for those listed below.

HPLC : Hewlett Packard 1090M  
Pump : Binary DR5  
Injector : Variable Volume Auto-injector  
UV Detector : HP 79880A Diode Array Detector  
Recorder : Kipp & Zonen Model BD 40  
Data System : HP 3357 LAS

Pre-column : Whatman Solvecon Silica Gel  
37-53 micrometre, 4.8mm x 100mm

Guard column : Waters Bondapak C18, 4mm x 23.1mm

Analytical : microBondapak C18, Waters Chrom.  
Div.,  
Column Millipore Div., 4mm x 300mm

Typical Column Pressure = 120 bar  
Initial Value of n = 8466

**Apparatus:** Analytical Balance, Sartorius Model 2462  
Pipets, Class A, 5mL, and 2mL  
Volumetric Flask, Class A, 100 mL  
Graduated Cylinder, Glass stopper, 2000mL  
Beakers, 50mL and 1000mL

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Effective Date: 10-18-91  
Page 2 of 7  
Written By: L.M.Rue

**TEST METHOD**

Vial, 8mL Wheaton 224804 or equivalent  
Caps, Wheaton 240509 or equivalent  
Vial, 1mL Wheaton 223682 or equivalent  
Aluminum seal, Teflon lined Wheaton 224211 or  
equiv.  
Filter, Millipore FHLP 013 00, 0.5 micrometre  
Filter, Millipore GSWP 047 00, 0.22 micrometre  
Filter, Millipore HATF 047 00, 0.45 micrometre  
Syringe, 10 mL Glass with Luer Lock tip  
Solid Phase Extraction Cartridge, SepPak C18 or  
equiv.

Instrument  
Parameters:

Detector : DAD UV  
Wavelength : A-210,4nm; B-210,4nm; Ref.-  
500,100nm  
Range : 200 - 300 nm  
Threshold : 5.0 mAU  
Peak Width : 0.400 min.  
Recorder : 10 mVFS  
Flow Rate : 1.0 mL/min.  
Column Press.: 120 bar is typical  
Chart Speed : 5 mm/min.  
Inj. Volume : 20 microliters  
Run Time : 16.0 min.

Reagents:

Sodium Perchlorate, Fisher S490, HPLC grade  
Methanol, HPLC grade, Burdick & Jackson #230  
Acetonitrile, HPLC grade, Burdick & Jackson #015  
Water, Milli-Q distilled, HPLC grade  
Phosphoric Acid, Reagent grade  
Octanoic acid, 99.5% purity, Aldrich 15,375-1  
Peroxyoctanoic acid, Ecolab prepared and  
purified

Preparation of  
Mobile Phase:

Mobile Phase - 0.1 M sodium perchlorate, pH 2.5  
in 60:40 , methanol:water

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LC Test Method No.: LC910300  
Name: Determination of  
Peroxyoctanoic and Octanoic Acids  
Supercedes: New  
Effective Date: 10-18-91  
Page 3 of 7  
Written By: L.M.Rue

#### TEST METHOD

1. Weigh 28.092g of sodium perchlorate into a tared 50 mL beaker.
2. Filter 1000 mL of Milli-Q water through a Millipore GSWP 0.22 micrometre filter with an all glass filter apparatus and sonicate for about 15 min. to degass.
3. Transfer the perchlorate to a 1000 mL beaker, using 400 mL of Milli-Q water to rinse the beaker.
4. Stir to dissolve the perchlorate.
5. Adjust pH to 2.5 with 1.0 N  $H_3PO_4$ . Initial pH is about 6.08.
6. Filter through a Millipore HATF 0.45 micrometre filter with an all glass filter apparatus.
7. Pour 800 mL into a 2000 mL grad. cylinder.
8. Add 1200 mL of filtered, degassed methanol and mix. Do not re-adjust the volume.
9. Pour into a LC reservoir and sonicate for 2 minutes.
10. Label with date and notebook reference.

#### Calibration:

##### Preparation of Standard of Peroxyoctanoic and Octanoic Acids

1. Weigh 0.016g of peroxyoctanoic acid and 0.160g of octanoic acid into a tared 100 mL volumetric flask.
2. Add acetonitrile:water, 60:40 to volume mark and mix to dissolve.
3. Filter about 3 mL through a FHL 0.5 micrometre filter collecting about 1 mL in each of three LC vials for the autosampler, and cap with a teflon lined aluminum seal. Label each vial on edge of the seal.
4. Calculate amount of each component in mg/mL using the purity of the component.

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Page 4 of 7  
Written By: L.M.Rue

## TEST METHOD

### Calibration with Standard

1. Enter the standard amounts for each compound as mg/mL into the calibration table of the method of the LAS data system.
2. Calibrate using the external standard, peak height method (HEST), with the HP 3357 IAS.
3. Perform 3 injections to equilibrate the system.
4. Using the LAS data system, calibrate with 3 injections of the standard using the CALBI and 2 CALBA commands. Then run an additional injection to verify the calibration.

### Sample Analysis:

#### Preparation of Samples

1. Weigh 0.10g of sample into a tared 8 mL vial.
2. Add by pipet 5.0 mL of Milli-Q water and mix.
3. Perform SepPak C18 extraction and clean-up of the sample as described in SepPak procedure.

#### SepPak Procedure

##### SepPak C18 Preparation

1. Remove plunger from a 10 mL glass syringe, and place the long end of a SepPak C18 cartridge on the Luer tip of the syringe barrel.
2. Pour 5 mL of acetonitrile into the syringe.
3. Insert the plunger and pump the acetonitrile through the SepPak C18 cartridge.
4. Remove the cartridge from the syringe tip.

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Page 5 of 7  
Written By: L.M.Rue

**TEST METHOD**

5. Repeat steps 1 through 4 with 10 mL of water and then 2 x 10 mL of air in place of the acetonitrile.

**Sample Application to SepPak**

1. Remove plunger from a 10 mL glass syringe, and place the long end of a SepPak C18 cartridge on the Luer tip of the syringe barrel.
2. Transfer sample from 8 mL vial to syringe quantitatively. Rinse the vial with 2.0 mL of water and add to the syringe.
3. Insert the plunger and pump the sample through the SepPak C18 cartridge slowly.
4. Remove the cartridge from the syringe tip.
5. Repeat steps 1 through 4 with 2.0 mL of water and then with 10 mL of air in place of the sample.

**Sample Removal from SepPak**

1. Remove SepPak C18 cartridge and plunger from the 10 mL glass syringe, and replace the long end of the SepPak cartridge on the Luer tip of the syringe barrel.
2. Pipet 2 mL of acetonitrile:water, 80:20 into the syringe.
3. Insert the plunger and pump the solvent through the SepPak C18 cartridge and collect eluate in a 2 mL volumetric flask to the volume mark.
4. Stopper the flask and mix. Transfer by means of a disposable pipet part of the sample solution into a 1 mL vial for the Auto-sampler.
5. Clean SepPak C18 cartridge with steps from SepPak C18 Preparation after each sample extraction.

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Page 6 of 7  
Written By: L.M.Rue

TEST METHOD

Analysis of Samples

1. Perform duplicate injections for each sample, obtaining the report from the HP LAS system to provide the amount in mg/mL.
2. Calculate the percent of peroxyoctanoic acid and octanoic acid in the sample for each injection.

$$\text{Percent} = ((\text{mg/mL found} \times 2 \text{ mL}) / \text{sample-wt., mg}) \times 100$$

3. Report the mean % Peroxyoctanoic acid and the mean % Octanoic acid found in the sample for the KX6049 sanitizer product.

Precision:

Precision Statements

Peroxyoctanoic Acid

1. The method precision = 2s based on the analysis of 5 replicates of the same sample on each of two days by the same analyst using the same instrument with duplicate injections was 0.0644 %. The mean concentration of the samples was 0.503 %.
2. The system precision = 2s based on 10 injections of the same sample solution by the same analyst using the same instrument on the same day was 0.0211 %. The measurements were made one after the other on a sample whose mean concentration was 0.465 %.

Octanoic Acid

1. The method precision = 2s based on the analysis of 5 replicates of the same sample on each of two days by the same analyst:

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Page 7 of 7  
Written By: L.M.Rue

TEST METHOD

using the same instrument with duplicate injections was 0.0977 %. The mean concentration of the samples was 3.3907 %.

2. The system precision = 2s based on 10 injections of the same sample solution by the same analyst using the same instrument on the same day was 0.0849 %. The measurements were made one after the other on a sample whose mean concentration was 3.3611 %.

References: Method LC9103

Prepared by: Larry M. Rue

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